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Certificate of Analysis

MSK2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-616, 14-616-K, 14-616M
Parent Lot # D7PN035U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, human MSK2, amino acids 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Activated using MAPK2 (cat# 14-173) and repurified using Ni^{2+}/NTA-agarose. Purity 64% by SDS-PAGE and Coomassie blue staining. MW = 89.9kDa.

Specific Activity (Parent lot# D7PN035U): 130U/mg, where one unit of MSK2, active activity is defined as 1nmol phosphate incorporated into 30µM modified Crosstide (GRPRTSSFAEGKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.9285mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 0.03% Brij-35, 270mM sucrose. Frozen solution.

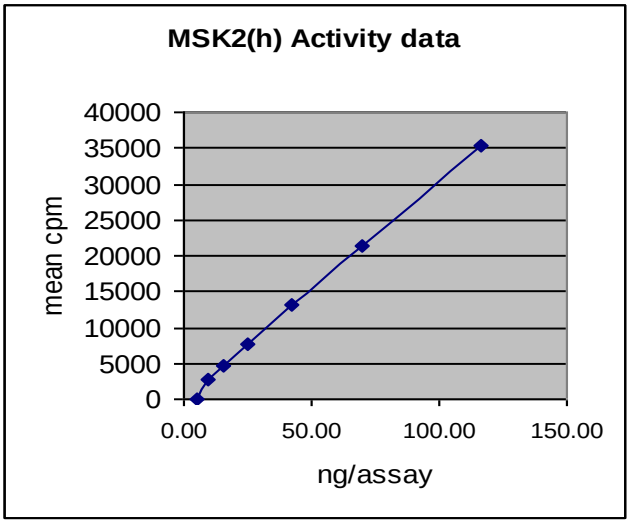
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

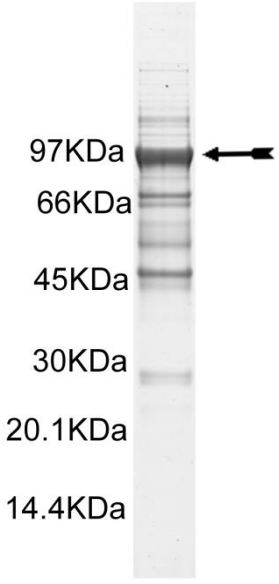
Quality Control Testing

Kinase Assay: 42–116ng of this lot of enzyme phosphorylated 30µM modified Crosstide (GRPRTSSFAEGKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as MSK2 with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MSK2, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Modified Crosstide (GRPRTSSFAEGKK):** Use a final assay concentration of 30µM. Make a 300µM stock. Add 2.5µl of stock per assay point.
3. **MSK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 42–116ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **Crosstide (GRPRTSSFAEGKK)**.
3. Add **2.5µl (42–116ng) MSK2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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MSK2 Sequence Information

Protein Human MSK2

Tags *N*-terminal 6His

Native sequence G37 of the recombinant protein is equivalent to G2 of human MSK2

Accession number GenBank AJ010119

Recombinant MSK2 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMGIRN SKAYVDGDED DDESCAVELR ITEANLTGHE
  61 EKVSVENFEL LKVLGTGAYG KVFLVRKAGG HDAGKLYAMK VLRKAALVQR AKTQEHTRTE
 121 RSVLELVRQA PFLVTLHYAF QTDAKLHLIL DYVSGGEMFT HLYQRQYFKE AEVRVYGGEI
 181 VLALEHLHKL GIIYRDLKLE NVLLDSEGHI VLTDFGLSKE FLTEEKERTF SFCGTIEYMA
 241 PEIIRSKTGH GKAVDWWSLG ILLFELLTGA SPFTLEGERN TQAEVSRRIL KCSPPFPPRI
 301 GPVAQDLLQR LLCKDPKKRL GAGPQGAQEV RNHPFFQGLD WVALAARKIP APFRPQIRSE
 361 LDVGNFAEEF TRLEPVYSPP GSPPPGDPRI FQGYSFVAPS ILFDHNNAVM TDGLEAPGAG
 421 DRPGRAAVAR SAMMQDSPFF QQYELDLREP ALGQGSFSVC RRCRQRQSGQ EFAVKILSRR
 481 LEANTQREVA ALRLCQSHPN VVNLHEVHHD QLHTYLVLEL LRGGELLEHI RKKRHFSESE
 541 ASQILRSLVS AVSFMHEEAG VVHRDLKPEN ILYADDTPGA PVKIIDFGFA RLRPQSPGVP
 601 MQTPCFTLQY AAPELLAQQG YDESCDLWSL GVILYMMLSG QVPFQGASGQ GGQSQAAEIM
 661 CKIREGRFSL DGEAWQGVSE EAKELVRGLL TVDPAKRLKL EGLRGSSWLQ DGSARSSPPL
 721 RTPDVLESSG PAVRSGLNAT FMAFNRGKRE GFFLKSVENA PLAKRRKQKL RSATASRRGS
 781 PAPANPGRAP VASKGAPRRA NGPLPPS
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Recombinant MSK2 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg gatccggaat tcaaaggcct acgtcgacgg ggacgaggac
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 181 gagaaggtga gcgtggagaa cttcgagctg ctcaaggtgc tgggcacggg agcctacggc
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1621 gcaagccaga tcctgcgcag cctcgtgtcg gccgtgagct tcatgcacga ggaggcgggc
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2401 aacggccccc tgcccccctc ctaa
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Reviewed and approved by site quality representative.